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Research Article

# Reproductive condition of an invasive snake in urban, savanna, and forest habitats

Andrew M. Durso[1*], Shane R. Siers[2*], Robert N. Reed[3,4], Julie A. Savidge[5]

1 *Florida Gulf Coast University, Fort Myers, Florida, USA*
2 *U.S. Department of Agriculture, Wildlife Services, National Wildlife Research Center, Barrigada, Guam, USA*
3 *U.S. Geological Survey, Fort Collins Science Center, Fort Collins, Colorado, USA*
4 *U.S. Geological Survey, Pacific Island Ecosystems Research Center, Hawaii National Park, Hawaii, USA*
5 *Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, Colorado, USA*
*Corresponding author: Andrew M. Durso (amdurso@gmail.com)*

## Abstract

Predation by Brown Treesnakes (*Boiga irregularis*) has caused the decline or extinction of all native forest vertebrates on Guam, including birds, lizards, and bats. Loss of the highest-quality prey has caused shifts in Brown Treesnake size and life history. We sought to understand how reproductive condition varies among habitats island-wide, particularly comparing urban and savanna habitats (where some primarily non-native large prey remain) with prey-depauperate forest habitats. We dissected 100 snakes from each of 18 sites stratified by 6 habitat types and calculated a body condition index, relative follicle size (for females; N = 817) and relative testes size (for males; N = 992) for each. Low detection of reproductive females continues to hamper research on their biology, as only 11% of females had secondary vitellogenic follicles (N = 89). Although variation among sites within habitats was high, above-average reproductive development of both males and females was most frequently observed in urban and savanna habitats, emphasizing the potential for prey subsidies in these habitats to fuel population growth and affect invasive snake management objectives. Furthermore, the trade-off between body condition and reproductive condition appeared to be more severe in forested habitats and less severe in urban habitats for both males and females. There were few obvious seasonal patterns, although there was a slightly lower probability of females having secondary vitellogenic follicles in the wet season compared to the dry season.

**Key words:** Brown Treesnake, Boiga irregularis, Guam, introduced, reproduction, body condition, season

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## Introduction

Brown Treesnakes (*Boiga irregularis*) (hereafter BTS) were accidentally introduced to Guam, most likely from Manus Island, Papua New Guinea, around the end of World War II (Richmond et al. 2015). Over the 70 years since BTS first became established on Guam, they have caused extinction of most of their preferred prey (birds), declines or loss of other native and non-native prey (lizards and mammals) (Savidge 1987; Wiles 1987; Fritts and Rodda 1998), and cascading effects on pollination, seed dispersal and forest composition (Mortensen et al. 2008; Rogers et al. 2017). BTS also regularly cause power outages and medically significant snakebites

* These authors contributed equally.

on Guam (Rodda and Savidge 2007). A desire to manage these problems and concern over the further spread of BTS has resulted in an extensive research and management program including efforts aimed at interdiction, suppression, and eradication (Brown Treesnake Technical Working Group 2015, 2020).

Loss of the highest-quality prey (*i.e.*, birds and mammals) has caused a shift in the average and maximum size of BTS on Guam, such that very large snakes are less frequently encountered (Savidge 1991), though whether this is due to plasticity, adaptation, or a combination is unknown. Seasonal variation in BTS reproduction is minimal in Guam's tropical climate (Mathies et al. 2010), in contrast to the discrete reproductive season of BTS in Australia (Shine 1991; Whittier and Limpus 1996; Bull et al. 1997). The role of spatial variation, particularly the potential for certain habitats to undermine BTS management objectives, is less clear. Understanding spatial variation in reproduction of female and male BTS of three demographic categories of management interest (juvenile, transitional, and mature, sensu Siers et al. 2017a) can be meaningful for the planning and execution of successful interventions.

We conducted robust island-wide sampling of BTS populations on Guam to evaluate differences in population characteristics such as size distributions and diet composition, sampling approximately 100 snakes from each of 18 sites stratified by habitat and season (Siers 2015; Siers et al. 2017a, b). Size distribution and body condition varied seasonally and among habitats, although the degree of variation among sites within habitats was high (Siers et al. 2017b). Upon necropsy of these ~1800 snakes, we also recorded the status of their gross reproductive organs to investigate ecological variation in reproductive status. Of particular interest are savanna and urban habitats, in which BTS body condition was more heterogeneous and where larger, more robust BTS, particularly males, were more prevalent compared to forest habitats, presumably due to availability of larger prey.

Here we use these data to explore and describe reproductive morphology in aseasonally-reproducing BTS. As stated in Rodda and Savidge (2007), "probably no aspect of Brown Tree Snake biology has been more frustrating to scientists than reproduction" due to the extreme difficulty of breeding BTS in captivity (Mathies and Miller 2003), complex role of pheromones in courtship (Murata et al. 1991), differences in seasonality of reproduction with the native range (Shine 1991; Whittier and Limpus 1996; Bull et al. 1997), and extremely low opportunistic encounter rate of wild clutches and probable use of tree cavities and deep underground crevices by ovipositing females (Savidge 1986; Ehmann 1993). Much of what is known about BTS reproduction comes from museum collections (Bull and Whittier 1996) or studies taking place in captivity (Barnett 1993; Greene and Mason 2000) where the stress of captivity has mostly overwhelmed attempts to use hormonal profiles to study reproductive phenology in the wild (Mathies et al. 2004; Aldridge and Arackal 2005; Waye 2007, but see Moore et al. 2005). As a result, aspects such as typical reproductive frequency, breeding seasonality, and clutch size have remained poorly understood. We evaluate whether BTS reproductive condition data obtained from this large sample on Guam might enlighten research and management efforts where other approaches have not. We ask whether gross reproductive morphology of males and females differed among sites, among habitats, or seasonally, with the expectation that differences among habitats in structure or resource availability might influence the reproductive development of BTS.

# Methods

## Field Methods

We used data from nocturnal visual surveys at 18 sites across the entire island of Guam, three located in each of six habitats, representing 95% of the terrestrial habitats on the island (Fig. 1; landcover data from Amidon et al. 2017). Sites were separated by at least 2 km and were selected to be dispersed across Guam and to contain large, representative tracts of their habitat type. We sampled each site at least twice during the wet season (June through November) and twice during the dry season (December through May), except for one limestone forest site that was sampled in one continuous effort due to impending construction. We conducted searches for approximately 4 hours after sunset, scanning habitat edges with powerful headlamps (Lupine Lighting Systems, Dresden, Germany) and hand-capturing any snakes encountered. Visual surveys yield samples that are more representative of the size distribution within a BTS population than does trapping (Rodda and Fritts 1992b; Rodda et al. 2007).

The six habitat types follow the categories of Mueller-Dombois and Fosberg (1998) and were defined using high-resolution data from Liu and Fischer (2005) and ground-truthed during site selection:

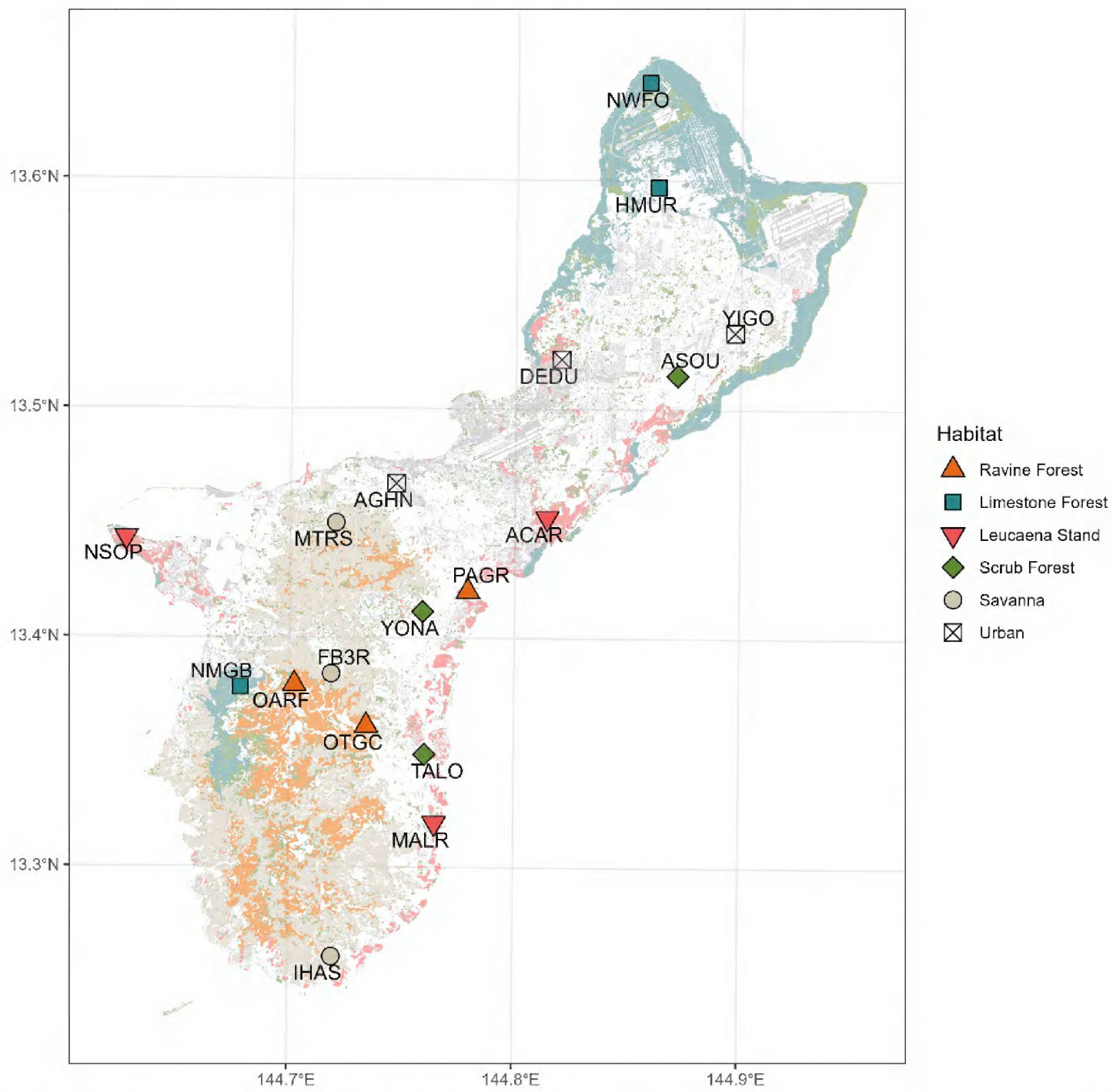


**Figure 1.** Map showing the 18 sites, shape- and color-coded by habitat, with Guam landcover shaded using the same six colors. Landcover from Amidon et al. 2017. All other landcover categories shown as white.

1. **Limestone forest** is found on elevated limestone plateaus and composed of moist, broadleaved evergreen forest of predominantly native species.
2. **Ravine forests** are low-lying areas bordering flowing and ephemeral rivers and are primarily moist green forests containing high proportions of palms, bamboos, and pandans (*Pandanus* spp.).
3. **Scrub forest** is variable and comprised primarily of secondary growth of non-native species on disturbed land.
4. **Leucaena stands** are primarily comprised of *Leucaena leucocephala*, an introduced species that was used to revegetate deforested areas on Guam.
5. **Savannas** are characterized by a mosaic of grassland interspersed with emergent shrubby vegetation and erosion scars.
6. **Urban areas** are diverse and include industrial, commercial, and residential areas. Areas sampled for this study were primarily residential.

A sample with a mean of 100 snakes (range = 99 to 104) was collected at each site during the period from 22 Mar 2010 to 27 Sep 2012, for a total of 1812 BTS (992 males and 817 females). Following the size criteria of Savidge et al. (2007), 38.2% of the snakes were juveniles (< 700 mm), 48.8% were transitional (700–910 mm for females; 700–940 mm for males), and 13.0% were mature (> 910 mm for females; > 940 mm for males) (8.3% of males, 4.7% of females).

Schema for pooling classifications of habitat types included grouping urban and non-urban habitats (UNU), grouping all forest habitats but keeping savanna and urban separate (FSU), grouping all forest habitats and all non-forest habitats (FNF), and keeping all six habitats separate (habitat) (Siers 2015). Instead of evaluating all 18 sites separately as a fixed effect, site was incorporated as a random explanatory factor.

## Dissection

After capture, snakes were transported to the U.S. Geological Survey Brown Treesnake Laboratory and Rapid Response Facility, where snout-vent length (SVL) and mass were measured. Snakes were then euthanized and necropsied. Sex was determined by examining reproductive organs. For males, we measured the length and width in mm of both testes using calipers. For females, we recorded the length of each secondary vitellogenic follicle (those ≥ 5 mm) and counted all visible follicles < 5 mm in length. Where intracoelomic fat bodies were visible, we excised them with forceps and weighed in g. All animal use was conducted in accordance with Colorado State University Institutional Animal Care and Use Committee (IACUC) Protocol #09-1436A.

## Body condition

Body condition was assessed using linear, quadratic, third-, fourth-, and fifth-order polynomial models fit to a regression of the log of snake body mass (minus the mass of any stomach contents) against the log of snake SVL (Jakob et al. 1996; Falk et al. 2017). Model selection was performed using Akaike information criterion (AIC) values. We scaled residuals from the top model so that values are in units of standard deviations from the predicted value. A snake with a body condition index (BCI) value of -0.5 was one-half of one standard deviation below the average body condition, and 95% of all snakes had BCI scores < +1.96 and > -1.96, with

a mean of zero. We examined residual plots to ensure homoscedasticity and independence from body length.

We calculated BCI separately for males and females. For analyses that used all individuals of a given sex, we denoted the body condition index $BCI_a$, whereas in analyses that considered only females with secondary vitellogenic follicles (N = 89) or males with two measurable testes (N = 519), we recalculated $BCI_e$ for that subset to keep the measure centered on 0.

## Female reproductive condition

We used three measures of female reproductive condition. These were:

1. The presence or absence of secondary vitellogenic follicles (≥ 5 mm in length);
2. The number of secondary vitellogenic follicles, adjusted for SVL using the same procedure used to calculate body condition (described above); and
3. The average length of secondary vitellogenic follicles, adjusted for SVL using the same procedure used to calculate body condition.

Because our captures varied so much in reproductive stage, we were unable to calculate total or relative clutch mass or volume or other common measures of reproductive investment used for species that reproduce in synchrony. Savidge et al. (2007) considered females sexually mature if oviductal eggs were present, ovarian follicles were ≥ 5 mm, or oviducts were thickened (per Shine 1987; Shine 1991).

## Male reproductive condition

Variation in testis size is less susceptible to sampling over a long time period than variation in follicle size. Thus, we calculated a testis size index (TSI) for males, removing the effect of body size on testis size in the same way as for BCI above. Testis volume was modeled as ovoid using

$$volume = \frac{4}{3}\pi \cdot length \cdot \left(width^2\right)$$

and volumes from the two testes were added together for a single measure of male reproductive investment. Only male snakes where both testes were located and measured (N = 530) were used in analyses of testis size. Male BTS with TSIs > 5.5 (N = 11) were omitted as outliers. Presence or absence of two measurable testes was used as a binary response variable in logistic models.

## Modeling

Females (N = 817) and males (N = 992) were analyzed separately. We expected BCI to have an effect on reproductive condition so we included it as an explanatory variable in all models ($BCI_a$ for logistic models and $BCI_e$ for linear models).

We used mixed logistic regression (function glmer in base R) to examine the effect of habitat type on the presence or absence of secondary vitellogenic follicles or of two measurable testes. We tested whether the four-habitat classification schema described above (UNU, FNF, FSU, and habitat), season (wet/dry), and interactions between habitat and season explained variation among sites. We did

not include interactions with $BCI_a$. Site was included as a random explanatory variable. We restricted logistic regression of females to only BTS above the lower end of mature sizes observed by Savidge et al. (2007; SVL ≥840 mm for females). To attain convergence, we used method='nlminb' from the optimx package (Nash and Varadhan 2011; Nash 2014).

We also used a linear mixed model (function lmer in package lme4) with follicle count (corrected for SVL), mean follicle length (only follicles ≥ 5 mm; corrected for SVL) or testis volume (corrected for SVL) as the continuous response variable, the four-habitat classification schema and their interactions with season plus an additive-only term for $BCI_e$ as fixed explanatory variables, and site as a random explanatory variable. We used all individuals regardless of body size, except for those that lacked two measurable testes or secondary vitellogenic follicles, in linear mixed models.

We also separately explored which of two different schema for seasons had greater explanatory power: two-level wet or dry season (Jun-Nov or Dec-May) and four-level low, rising, high, and falling rainfall (Feb-Apr, May-Jul, Aug-Oct, and Nov-Jan) (refer to Suppl. material 1). The two-level wet/dry season variable outperformed the four-level season variable in all cases for females and more than half the time for males, so for simplicity we used two-level wet/dry season in all analyses.

Normality of residuals was checked using the shapiro.test function in base R. Where transformation was inadequate to achieve normality of residuals, we used non-parametric tests where possible. Model selection was performed using the model.sel function from package MuMIn (Bartón 2020) to rank models by AIC, and Tukey's pairwise post-hoc comparisons were calculated using the function emmeans in package emmeans. Fit of logistic models was assessed using QQ plots, residual plots, and tests for zero-inflation from package DHARMa (Hartig et al. 2024). Data used in this paper are available in Siers et al. (2025).

## Results

### Females

Of a total of 817 female BTS dissected across all sites, 339 contained follicles of any size, and 89 contained secondary vitellogenic follicles (follicles ≥ 5 mm in length). Female BTS with any follicles were larger (mean ± SD SVL = 950 ± 132 mm) than those without (650 ± 123 mm), and female BTS with secondary vitellogenic follicles were larger (1061 ± 110 mm) than those with only small (910 ± 115 mm) or no follicles (650 ± 123 mm). The smallest female containing follicles measured 543 mm SVL. The smallest female with secondary vitellogenic follicles measured 883 mm SVL. The largest female that lacked even small follicles measured 1064 mm SVL (Fig. 2). Five females >1200 mm SVL without secondary vitellogenic follicles (N = 2 in forest and 3 in savanna; no such snakes were encountered in urban habitats) are likely to have recently laid eggs. These 5 females had among the highest counts of unenlarged follicles, suggesting that they had already begun to yolk up their next clutch (ranks 1, 2, 6, 7, and 17 out of 339 females with follicles <5mm, and 1, 2, 3, 4, and 6 out of 13 females with SVL >1200mm). Omitting the five females >1200 mm SVL without secondary vitellogenic follicles from the dataset did not change model selection results.

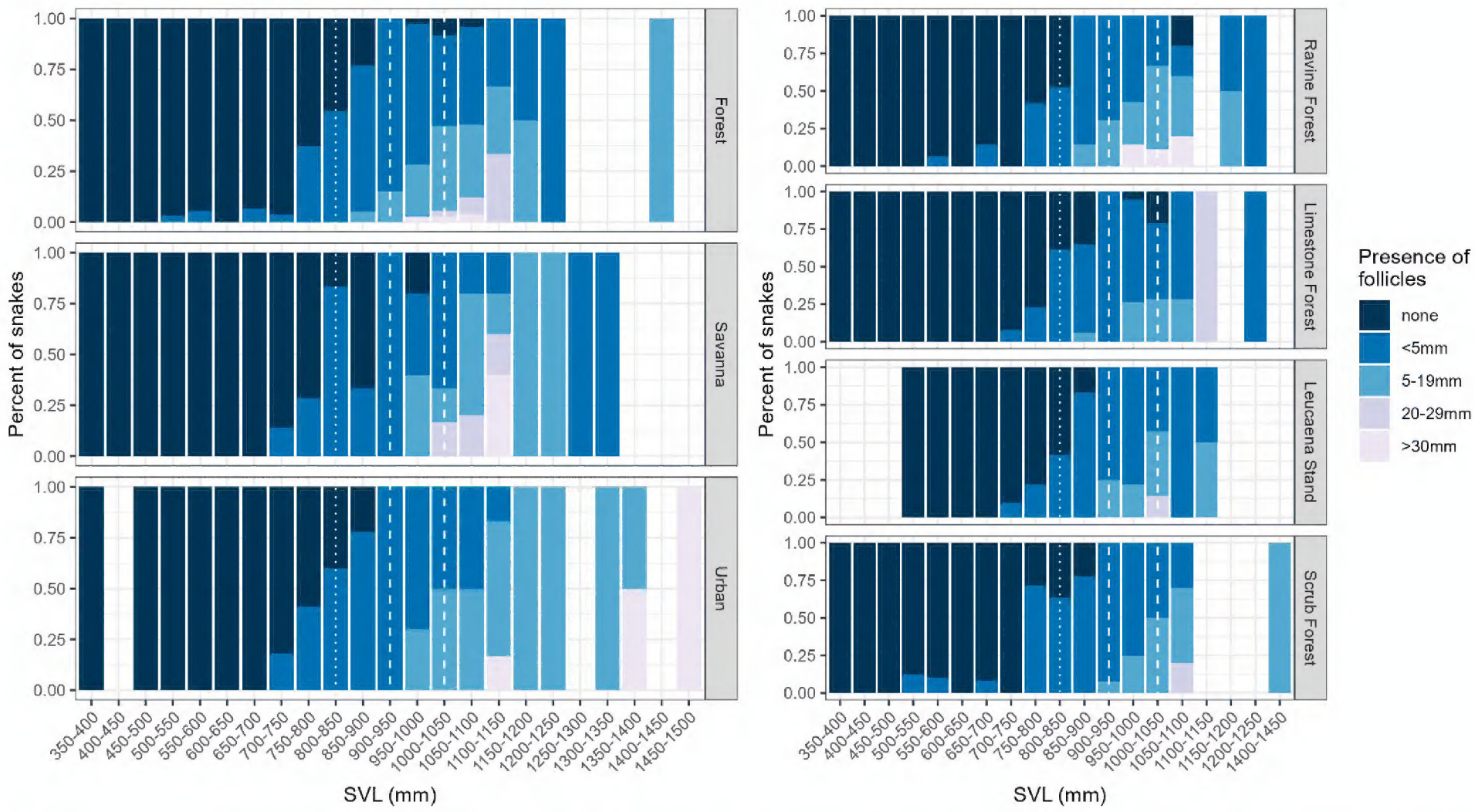


**Figure 2.** Percent of female *Boiga irregularis* of different sizes lacking follicles (darkest blue) and with follicles of different sizes (lighter shades of blue) among habitats. Left panel (top to bottom): forest, savanna, urban habitat. Right panel: the four forest types separated. Vertical dotted lines: minimum SVL of reproductive female from Savidge et al. 2007. Vertical dashed lines: mature female SVL range from Savidge et al. 2007.

Clutch size (counting only secondary vitellogenic follicles ≥ 5 mm in length) ranged from 1–14, with an interquartile range of 3–6, mean ± S.D. of 4.4 ± 2.4, and median of 4 (N = 89; Suppl. material 1: fig. S1A). When only females with follicles ≥20 mm in length were considered (N = 15), clutch size ranged from 3–14, with an interquartile range of 4–6, mean ± S.D. of 5.7 ± 2.6, and median of 5.

Mean follicle length (counting only secondary vitellogenic follicles ≥ 5 mm in length) ranged from 5.1 to 40.1 mm, with an interquartile range of 6.3–10.1, mean ± S.D. of 10.8 ± 8.0, and median of 7.5 (N = 89; Suppl. material 1: fig. S1B). For females with more than one follicle ≥ 5 mm in length (N = 76), the within-clutch standard deviation averaged 2.7 mm and was less than the mean in every case (Suppl. material 1: fig. S1B).

## Seasonal Variation

In the dry season, 48% (51/106) of mature females (>900 mm SVL) had secondary vitellogenic follicles, compared with just 30% (36/119) of mature females in the wet season. Based on the distribution of follicle lengths throughout the year by habitat, reproduction might be more seasonal in some habitats than others (Suppl. material 1: table S1). Females were captured in all habitats in at least eight of the twelve months. Only in urban areas did at least one female have secondary vitellogenic follicles in all sampled months (no urban female captures in October or November). Of months with at least 10 female BTS captures, females with secondary vitellogenic follicles made up ≥20% of captures in limestone forest in May, in ravine forest in December, in savanna in March, in scrub forest in August, and in urban areas in January and April (Suppl. material 1: table S1).

## Variation in Body Condition

BCI was highest in urban and lowest in forest habitats (Fig. 3A, B). Female BCI was significantly lower in limestone and ravine forest than in urban habitat (Suppl. material 1: fig. S2). BCI did not differ between wet and dry seasons in any habitat (Fig. 4 and Suppl. material 1: fig. S2; Kruskal-Wallis $p > 0.10$) except for urban (Kruskal-Wallis $X^2_1 = 6.73$, $p = 0.009$). Most females with secondary vitellogenic follicles in limestone forest, Leucaena stand and scrub forest had below-average BCI, and most females with secondary vitellogenic follicles in savanna had above-average BCI (Fig. 5).

## Model Selection

In the logistic model set where presence of secondary vitellogenic follicles is the response variable, site is a random effect, $BCI_a$ is a fixed effect present in all models, and only female BTS larger than the minimum mature size (≥840 mm) were included (N = 304), the top model included the fixed effect of season (weight = 0.31), the second-best model included an interaction between season and the forest-nonforest (FNF) habitat classification scheme (weight = 0.18), and the third-best model included additive effects of season and FNF (weight = 0.12) (Suppl. material 1: table S2). Other models that were >2 ΔAIC units from the top model but still above the null model were urban-nonurban (UNU) + season (weight = 0.11) and UNU*season (weight = 0.08).

In all logistic models, there was a consistent positive effect of $BCI_a$. In the top model, secondary vitellogenic follicles were more likely to be present in the dry season than in the wet season (Fig. 6). Because of the presence of interactions and

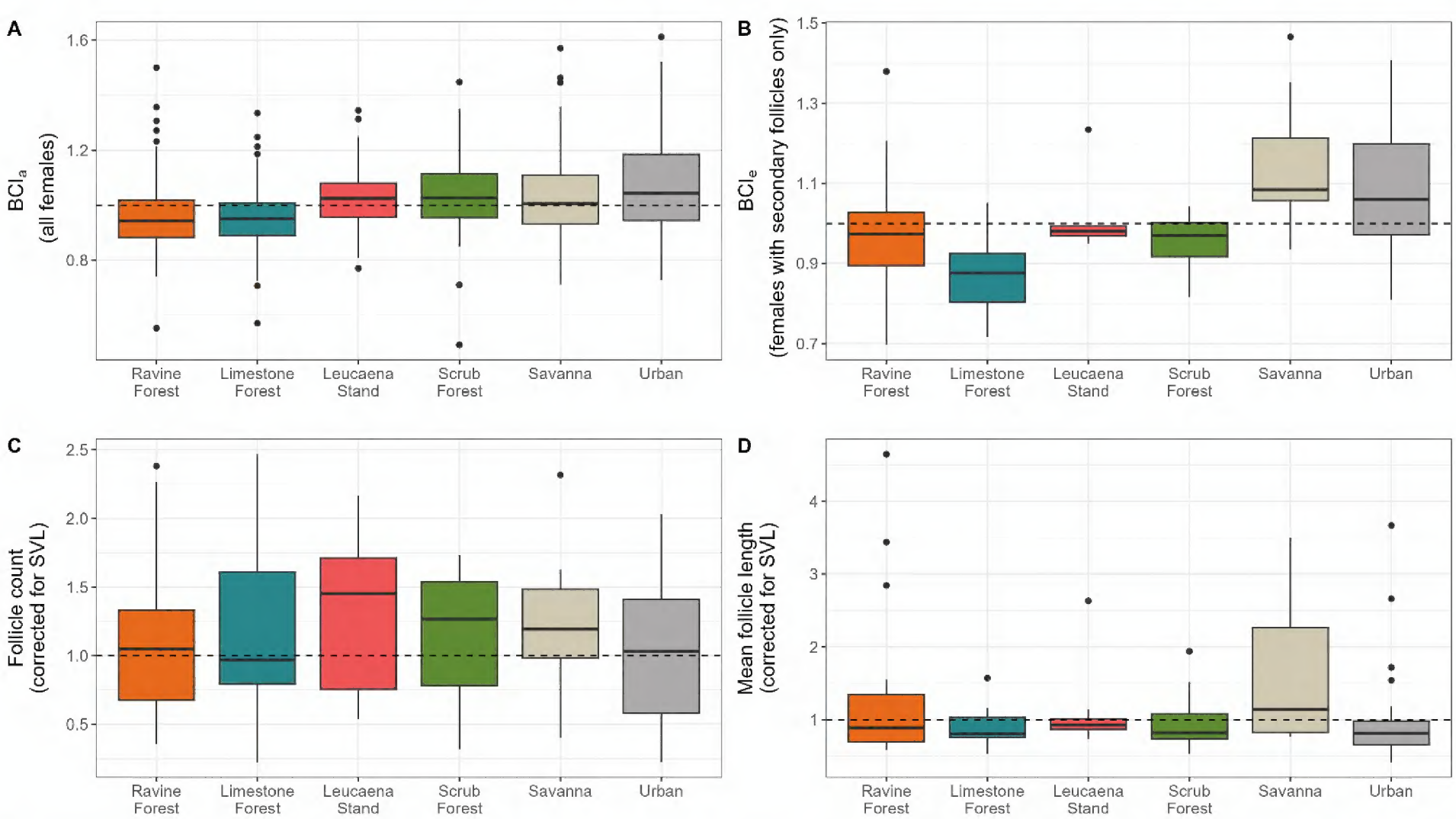


**Figure 3.** Variation among habitats in **A.** *Boiga irregularis* body condition index (BCI) calculated for all females (N = 817); **B.** BCI calculated for only females with secondary vitellogenic follicles (≥5 mm) (N = 89); **C.** Secondary vitellogenic follicle count (corrected for SVL); and **D.** Mean secondary vitellogenic follicle length (corrected for SVL). Horizontal dashed lines at 1 indicate the mean. Units of the y-axis are standard deviations.

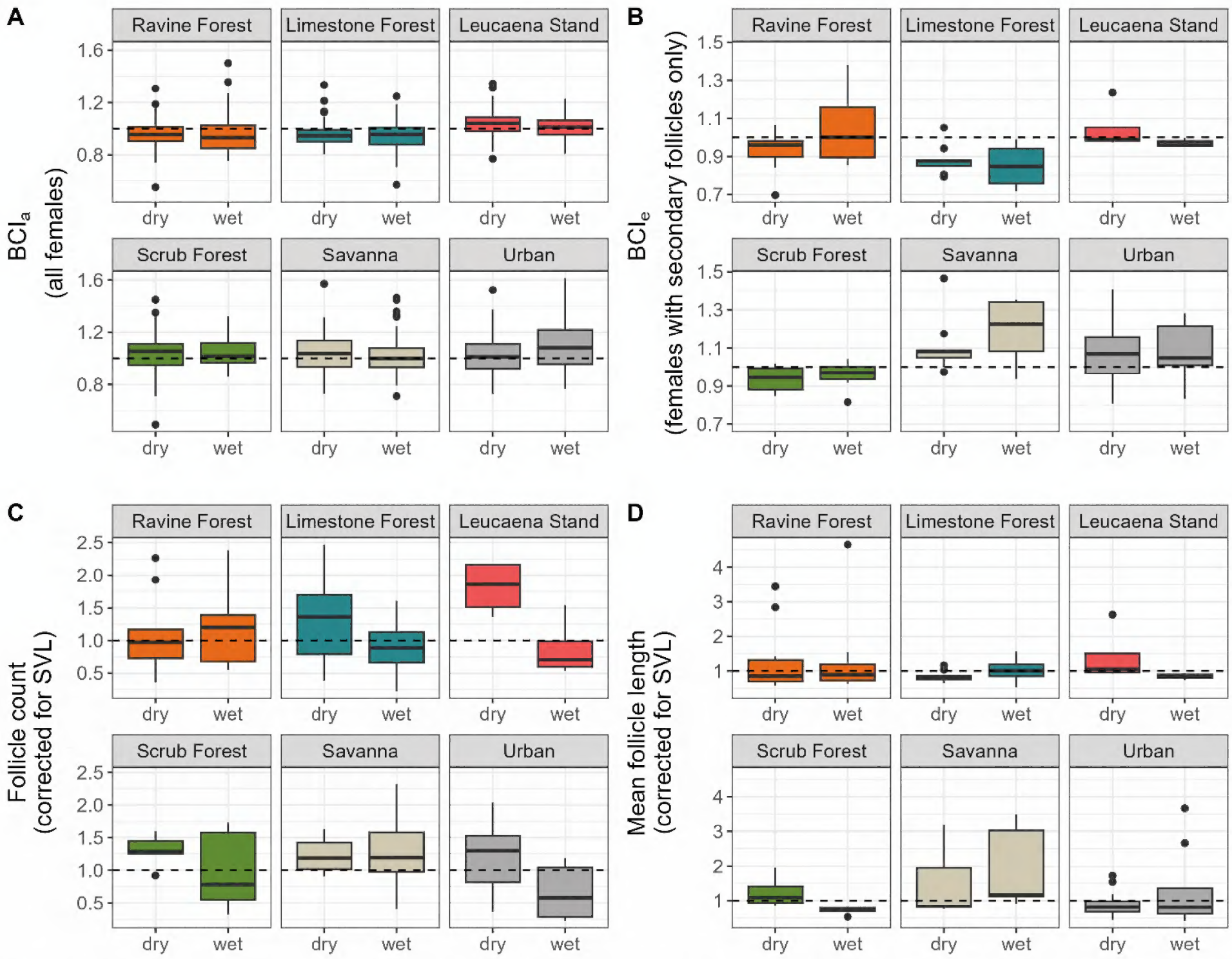


**Figure 4.** Variation between seasons and among habitats in **A.** *Boiga irregularis* body condition index ($BCI_a$) calculated for all females (N = 817); **B.** Body condition index ($BCI_e$) calculated for only females with secondary vitellogenic follicles (≥5 mm) (N = 89); **C.** Secondary vitellogenic follicle count (corrected for SVL); and **D.** Mean secondary vitellogenic follicle length (corrected for SVL). Horizontal dashed lines at 1 indicate the mean. Units of the y-axis are standard deviations.

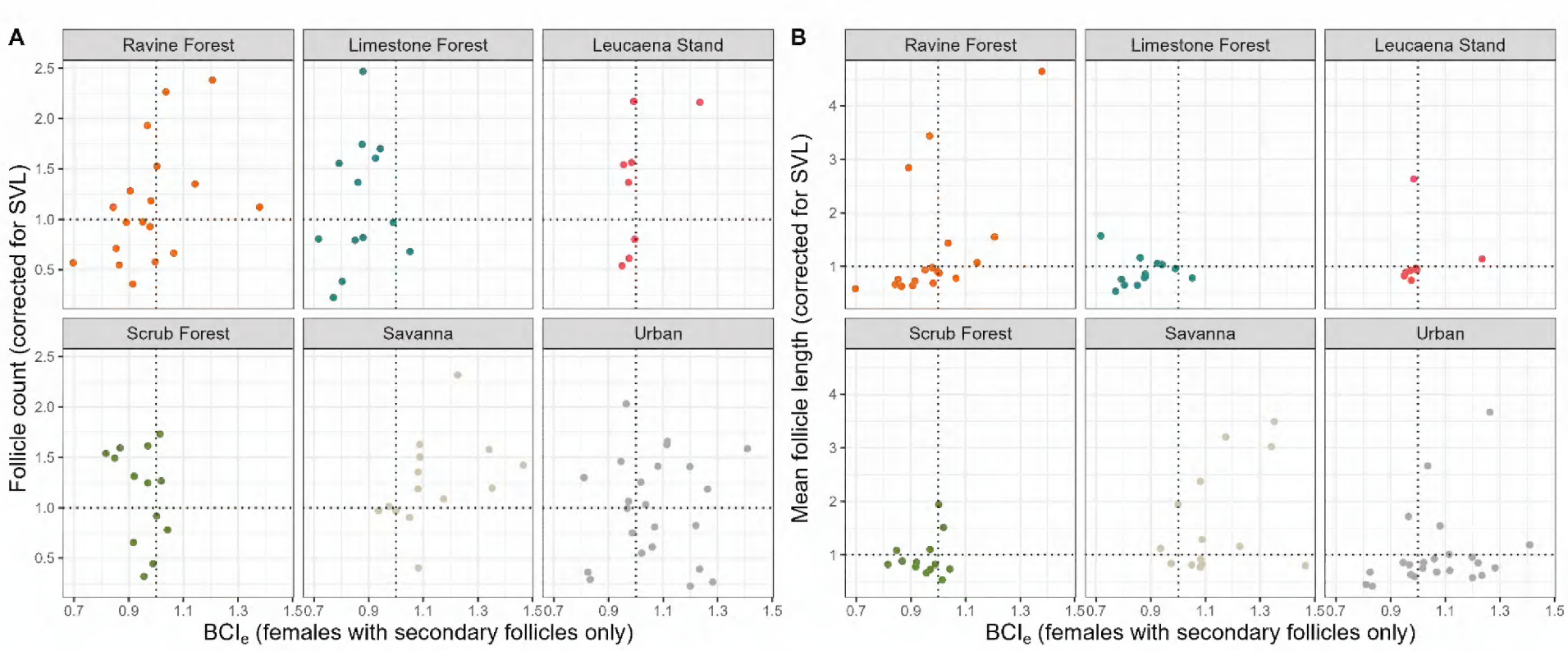


**Figure 5.** Scatterplot of body condition index ($BCI_e$) of female *Boiga irregularis* with secondary vitellogenic follicles (≥5 mm) (N = 89) against. **A.** Follicle count (corrected for SVL) and **B.** Mean follicle length (corrected for SVL) in six habitats on Guam. Dotted lines are means, units of axes are standard deviations.

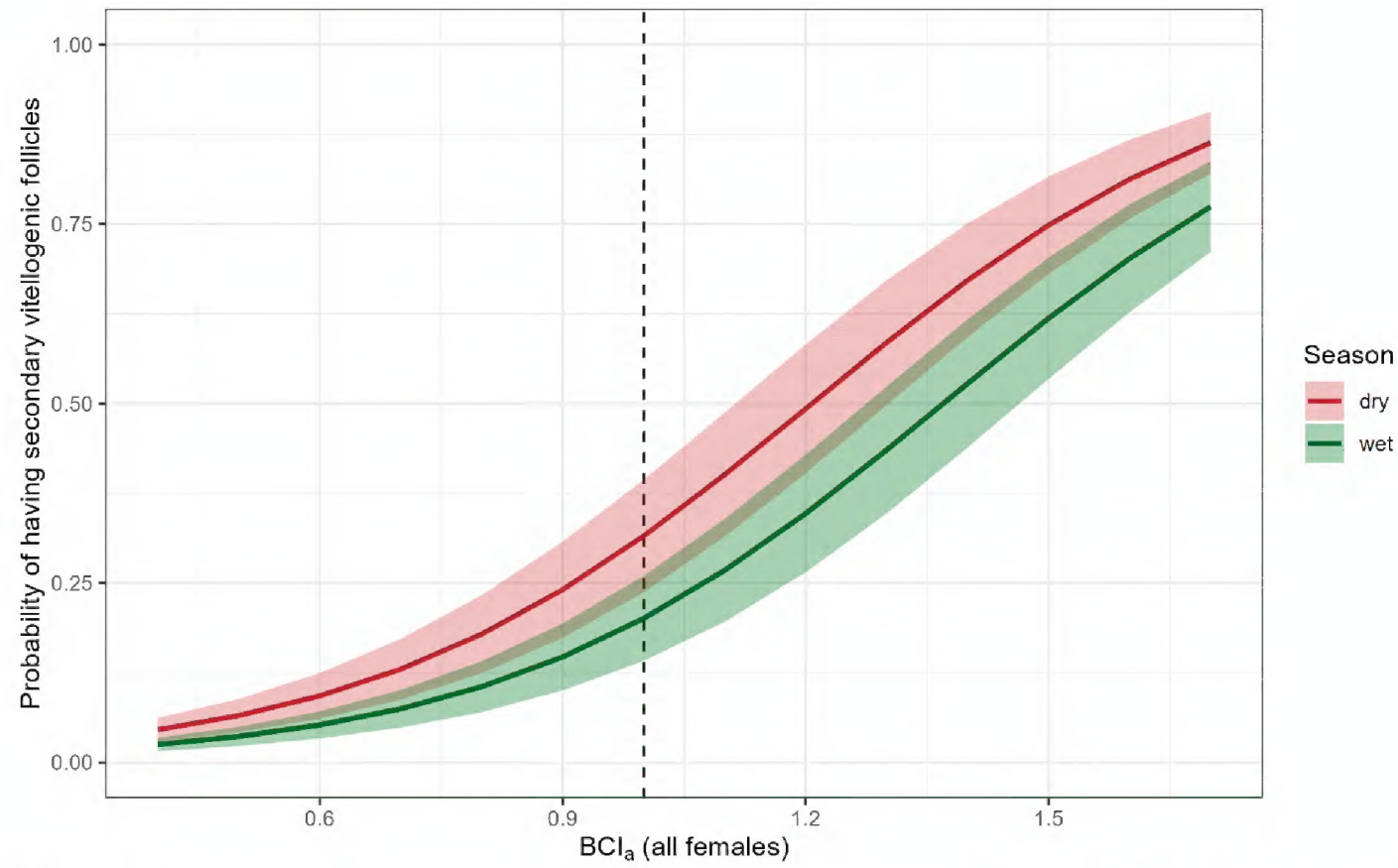


**Figure 6.** Relationship predicted from top mixed logistic model between $BCI_a$ and probability of enlarged (≥5 mm) follicles being present in female *Boiga irregularis* in wet and dry seasons.

the non-independence of FNF and UNU, we could not perform model averaging. Model fit for the top model was excellent (Suppl. material 1: fig. S3).

In the linear mixed model where the secondary vitellogenic follicle count (corrected for SVL) is the response variable, site is a random effect, and $BCI_e$ is a fixed effect present in all models, just 89 females had secondary vitellogenic follicles and could be included. The top model included the urban-nonurban (UNU) habitat classification scheme and season without an interaction (weight = 0.24), the second-best model included FNF + season (weight = 0.21), the third-best model included only season (weight = 0.19) and the fourth-best model included an interaction between season and UNU (weight = 0.10). All other models were >2 ΔAIC units from the top model and had less weight than the null model (Suppl. material 1: table S3). Model fit for the top model was good (Suppl. material 1: fig. S4), although some models had singular fits.

In the linear mixed model where the mean follicle length (corrected for SVL) is the response variable, site is a random effect, and $BCI_e$ is a fixed effect present in all models, just 89 females had secondary vitellogenic follicles and could be included. The top model was the null model with just $BCI_e$ (weight = 0.37). The UNU habitat classification scheme was the only other model within 2 ΔAIC units of the top model (weight = 0.32) (Suppl. material 1: table S4). Model fit for the top model was relatively poor (Suppl. material 1: fig. S5).

In all linear models, there was a consistent positive effect of $BCI_e$ (Fig. 7). Follicle counts were higher in non-urban habitats than in urban habitats (for a given $BCI_e$), and higher in the dry season than in the wet season (Fig. 7A).

## Odds ratios and effect sizes

For a one standard deviation increase in BCI, we expect a 287% increase in the odds of a female having secondary vitellogenic follicles (95% confidence interval 32% to 1052%). For a given BCI, the odds of secondary vitellogenic follicles being present decrease 54% (95% CI 31–93%) in the wet season compared to the dry season (Table 1; Fig. 6).

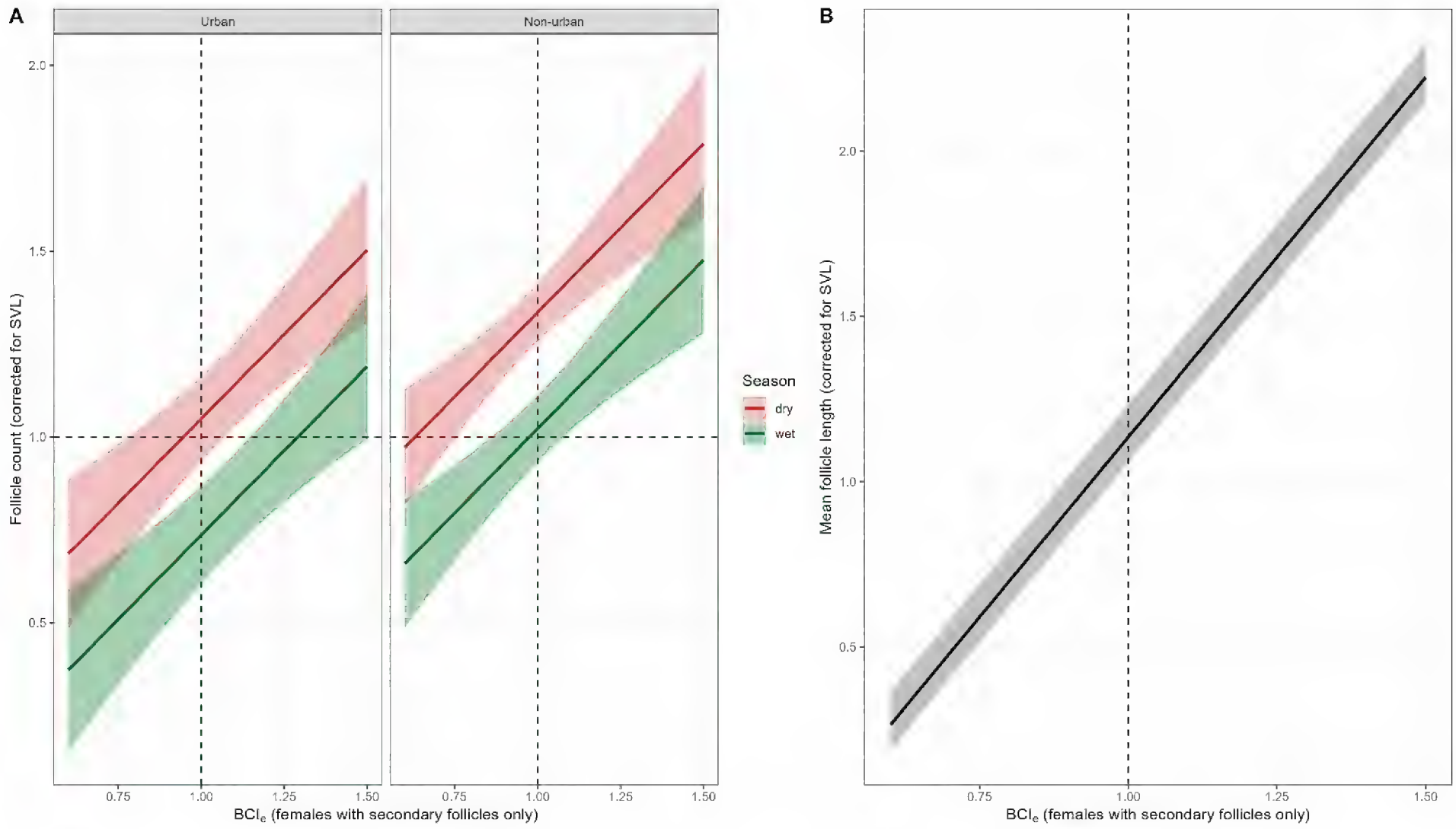


**Figure 7.** Predicted relationships from top linear mixed models between $BCI_e$ and **A.** Follicle count (corrected for SVL) between seasons and urban and non-urban habitats and **B.** Mean follicle length (corrected for SVL) in female *Boiga irregularis*.

**Table 1.** Odds ratios for top logistic models. The logistic model for males includes an interaction between habitat and season so there are five odds ratios ranging from 0.29 to 3.00 for the five non-intercept levels of habitat (the intercept is dry season ravine forest). Because site is a random factor, values of the intercept differ for each of the 18 sites; see supplement for full model output.

| Sex | Response variable | Top model | Weight | Odds ratio [LCI,UCI 95%] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | BCI | Habitat scheme | | | | | Season |
| Females | Presence of secondary vitellogenic follicles | $BCI_a$+season+(1\|site) | 0.31 | 46.4 [8.18, 263.4] | – | | | | | 0.54 [0.31,0.93] |
| Males | Presence of two measurable testes | $BCI_a$+habitat*season+(1\|site) | 0.97 | 4.29 [1.46, 12.96] | limestone forest | Leucaena stand | scrub forest | savanna | urban | 1.21 [0.59, 2.45] |
| | | | | | 0.29 [0.11, 0.70] | 1.29 [0.57, 2.84] | 1.68 [0.27, 1.85] | 1.37 [0.51, 3.94] | 3.00 [1.24, 7.24] | |

**Table 2.** Regression coefficients for top linear models. FNF = forest/non-forest; UNU = urban/non-urban.

| Sex | Response variable | Top model | Weight | Regression coefficient ± S.E. | | |
|---|---|---|---|---|---|---|
| | | | | BCI | Habitat scheme | Season |
| Females | Follicle count (corrected for SVL) | $BCI_e$+UNU+season+(1\|site) | 0.24 | 0.91 ± 0.36 | -0.28 ± 0.13 | -0.31 ± 0.11 |
| | Mean follicle length (corrected for SVL) | $BCI_e$+(1\|site) | 0.37 | 2.37 ± 0.52 | – | – |
| Males | Est. testes volume (corrected for SVL) | $BCI_e$+FNF+season+(1\|site) | 0.33 | 1.11 ± 0.30 | -0.20 ± 0.08 | -0.42 ± 0.13 |

A one standard deviation increase in BCI was predicted to correspond to an increase (± S.E.) of 0.91 (± 0.36) secondary vitellogenic follicles. For a given BCI, females at non-urban sites had 0.28 (± 0.13) more follicles than those at urban sites, and females in the dry season had 0.31 (± 0.11) more follicles than in the wet season (Table 2). A one standard deviation increase in BCI was predicted to correspond to an increase of 2.37 mm (± 0.52 mm) in average follicle length (Table 2).

## Males

Of a total of 992 male BTS dissected across all sites, 639 contained at least one measurable testis and 530 contained two measurable testes. Male BTS with two testes were larger (mean ± SD SVL = 948 ± 258 mm) than those with one (727 ± 131 mm) or zero (627 ± 129 mm). The smallest male with two measurable testes measured 436 mm, and the five largest males lacking measurable testes measured 1104, 1056, 980, 956, and 913 mm SVL. Estimated testes volume for males with two measurable testes (11 outliers >5.5 standard deviations from the mean omitted) ranged from 0.9 to 14,084 $mm^3$, with an interquartile range of 73–1092, mean ± S.D. of 1403 ± 2623 and median of 198 (N = 519). The anterior right testis was consistently longer and narrower than the posterior left testis; this asymmetry is widespread in snakes (Fox 1977).

## Variation in body condition

Male BCI was highest in urban and lowest in forest habitats (Figs 8, 9); BCI was significantly higher in urban habitat compared to ravine forest, limestone forest, and scrub forest (Suppl. material 1: fig. S2). BCI did not differ between wet and dry seasons in any habitat (Kruskal-Wallis $p > 0.10$) except for Leucaena stand (Kruskal-Wallis $X^2_1 = 5.73$, $p = 0.016$). Most males with two measurable testes present in limestone forest and ravine forest had below-average BCI (Fig. 10).

## Seasonal variation

Male BTS were most likely to have detectable testes in March (the driest month), November, and December (at the end of the rainy season/beginning of the dry season) (Suppl. material 1: table S5).

## Model Selection

In the logistic model set where presence of two measurable testes is the response variable, site is a random effect, and $BCI_a$ is a fixed effect present in all models (N = 979), the top model included the habitat classification scheme with all six habitats and an interaction with season, with a weight of 0.97. No other models were within 8 ΔAIC units of the top model (Suppl. material 1: table S6).

In all logistic models, there was a consistent positive effect of $BCI_a$, and two measurable testes were more likely to be present in the wet season than in the dry season in two habitats (limestone forest, Leucaena stand) but equally likely to be

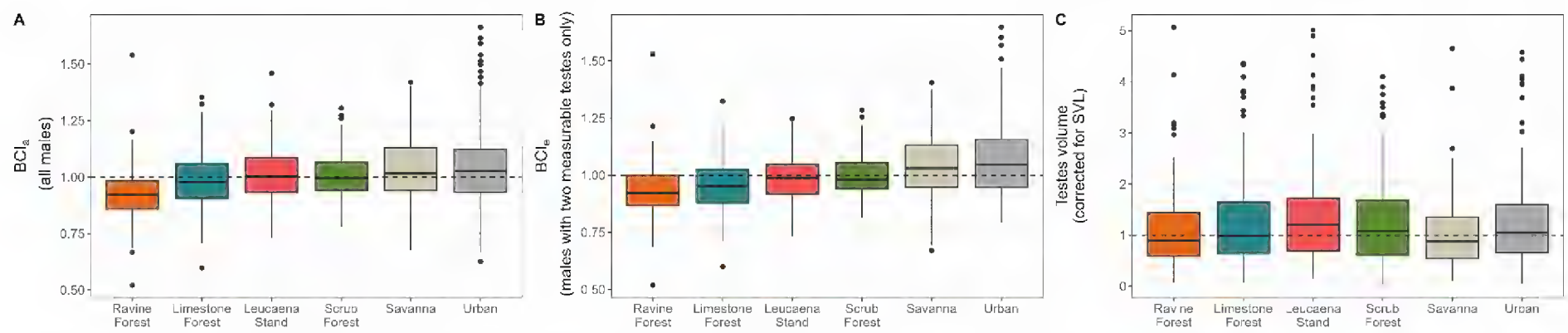


**Figure 8.** Variation among habitats in **A.** *Boiga irregularis* body condition index ($BCI_a$) calculated for all males (N = 979); **B.** $BCI_e$ calculated for only males with two measurable testes (N = 519); and **C.** Testes volume (corrected for SVL). Horizontal dashed lines at 1 indicate the mean. Units of the y-axis are standard deviations.

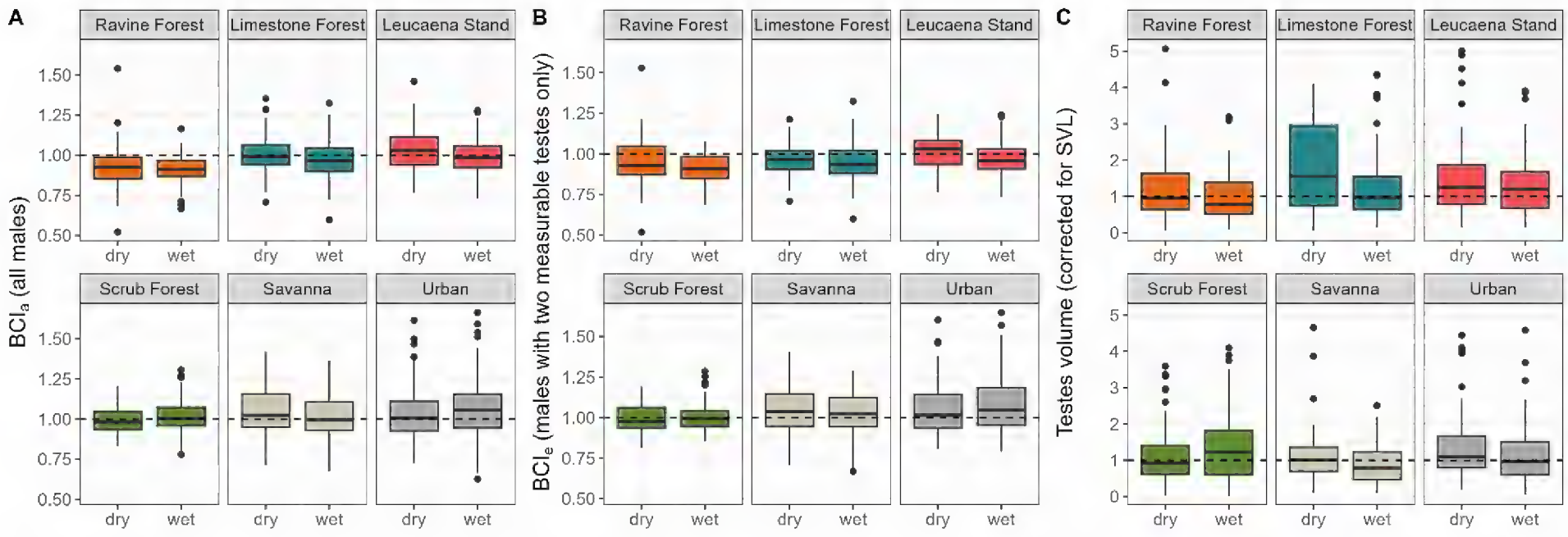


**Figure 9.** Variation among habitats and seasons in **A.** *Boiga irregularis* body condition index ($BCI_a$) calculated for all males (N = 979); **B.** $BCI_e$ calculated for only males with two measurable testes (N = 519); and **C.** Testes volume (corrected for SVL). Horizontal dashed lines at 1 indicate the mean. Units of the y-axis are standard deviations.

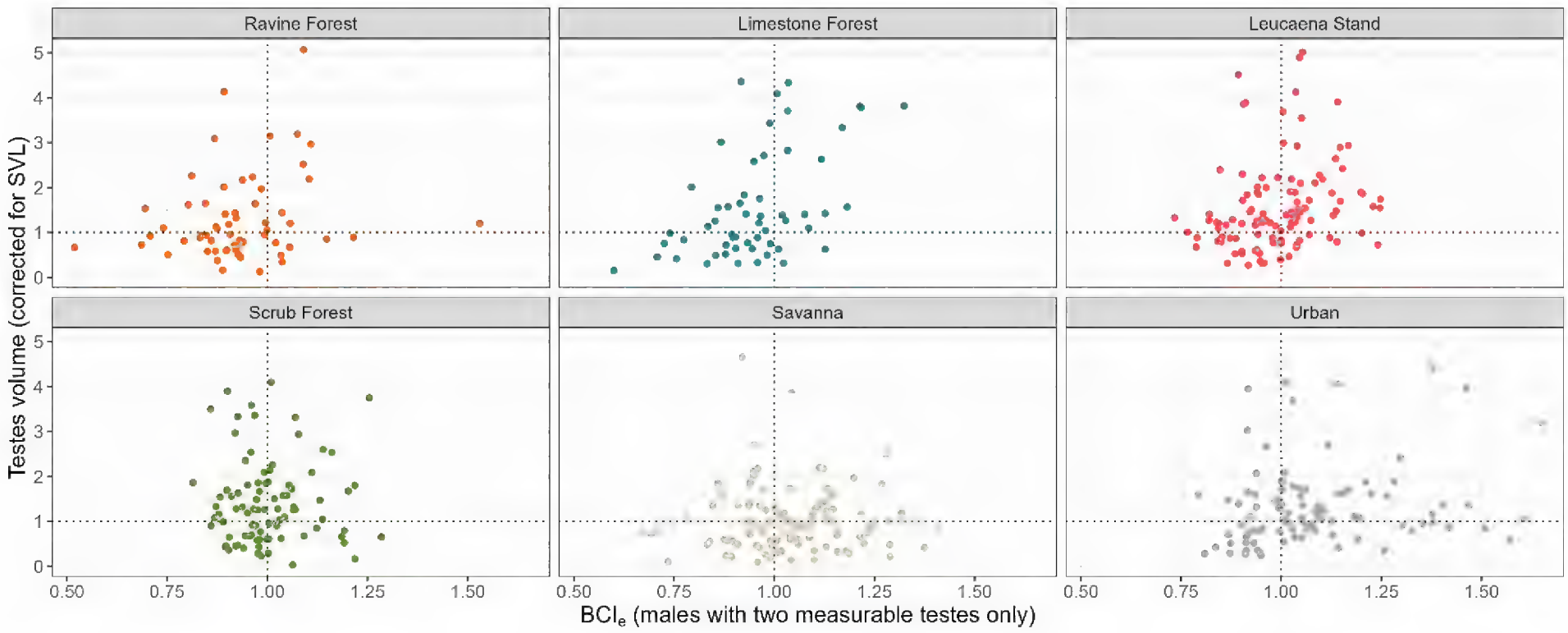


**Figure 10.** Scatterplot of body condition index ($BCI_e$) of male *Boiga irregularis* with two measurable testes (N = 519) against estimated total testis volume (corrected for SVL) in six habitats on Guam. Dotted lines are means, units of axes are standard deviations.

present in both seasons in the other four habitats (Fig. 11). Model fit for the top model was excellent (Suppl. material 1: fig. S6).

In the linear mixed model where the estimated volume of both testes (corrected for SVL) is the response variable, site is a random effect, and $BCI_e$ is a fixed effect present in all models, 519 males had two measurable testes and could be included. The top model included the forest-nonforest (FNF) habitat classification scheme and season without an interaction (weight = 0.33), the second-best model included FNF without season (weight = 0.27), and the third-best model included the forest-savanna-urban (FSU) habitat classification scheme (weight = 0.13). Other models that were >2 ΔAIC units from the top model but had more weight than the null model were FSU without season (weight = 0.11), FNF*season (weight = 0.05) and season without any habitat scheme (weight = 0.04) (Suppl. material 1: table S7). Model fit for the top model was poor (Suppl. material 1: fig. S7).

In all linear models, there was a consistent positive effect of $BCI_e$ on testes volume. In the top model, the estimated volume of both testes (corrected for SVL) was higher in forest habitats than in non-forest habitats (for a given $BCI_e$) and slightly higher in the dry season than in the wet season (Fig. 12).

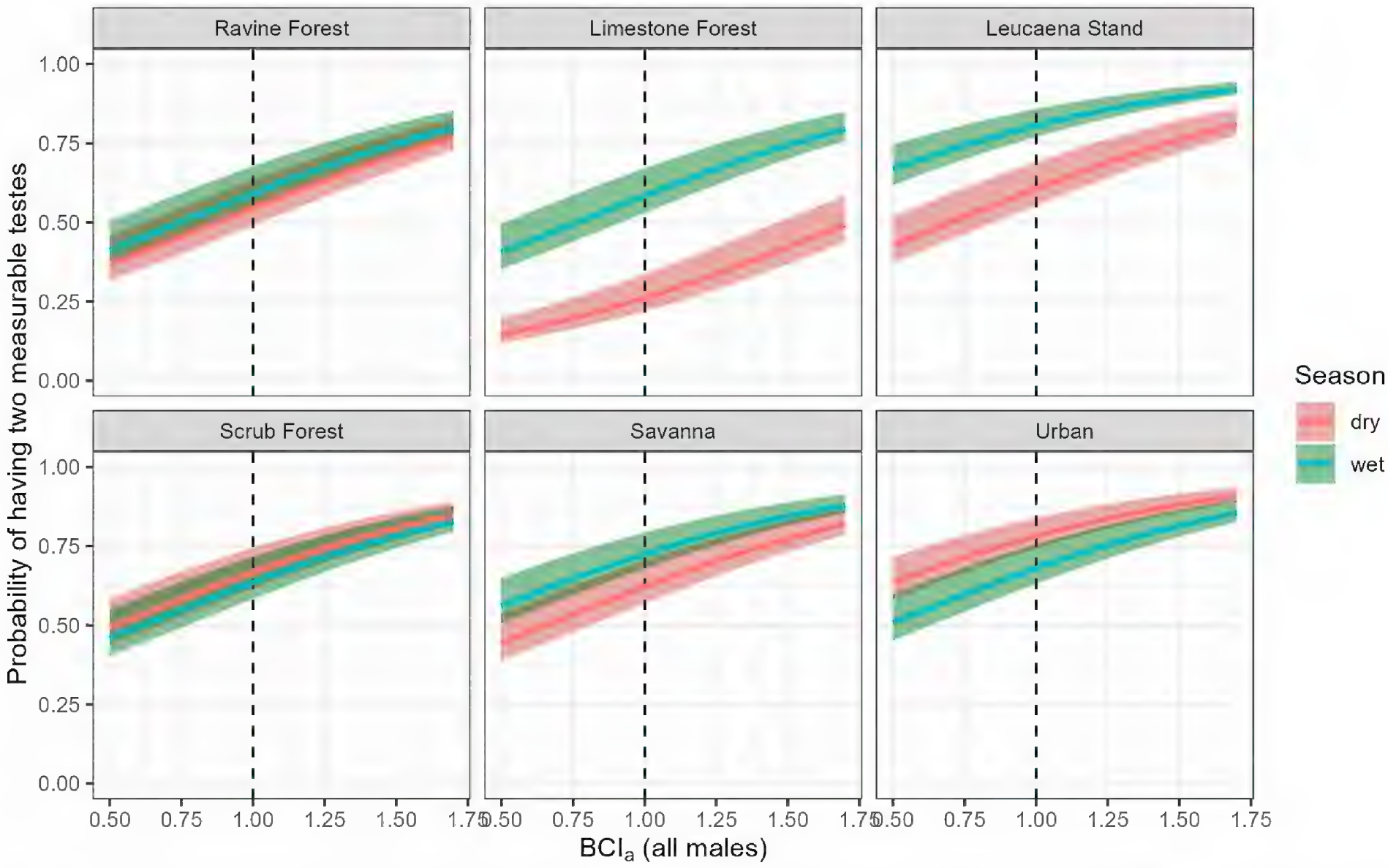


**Figure 11.** Predicted relationships from top mixed logistic model between $BCI_a$ and probability of male *Boiga irregularis* having two measurable testes among habitats and seasons.

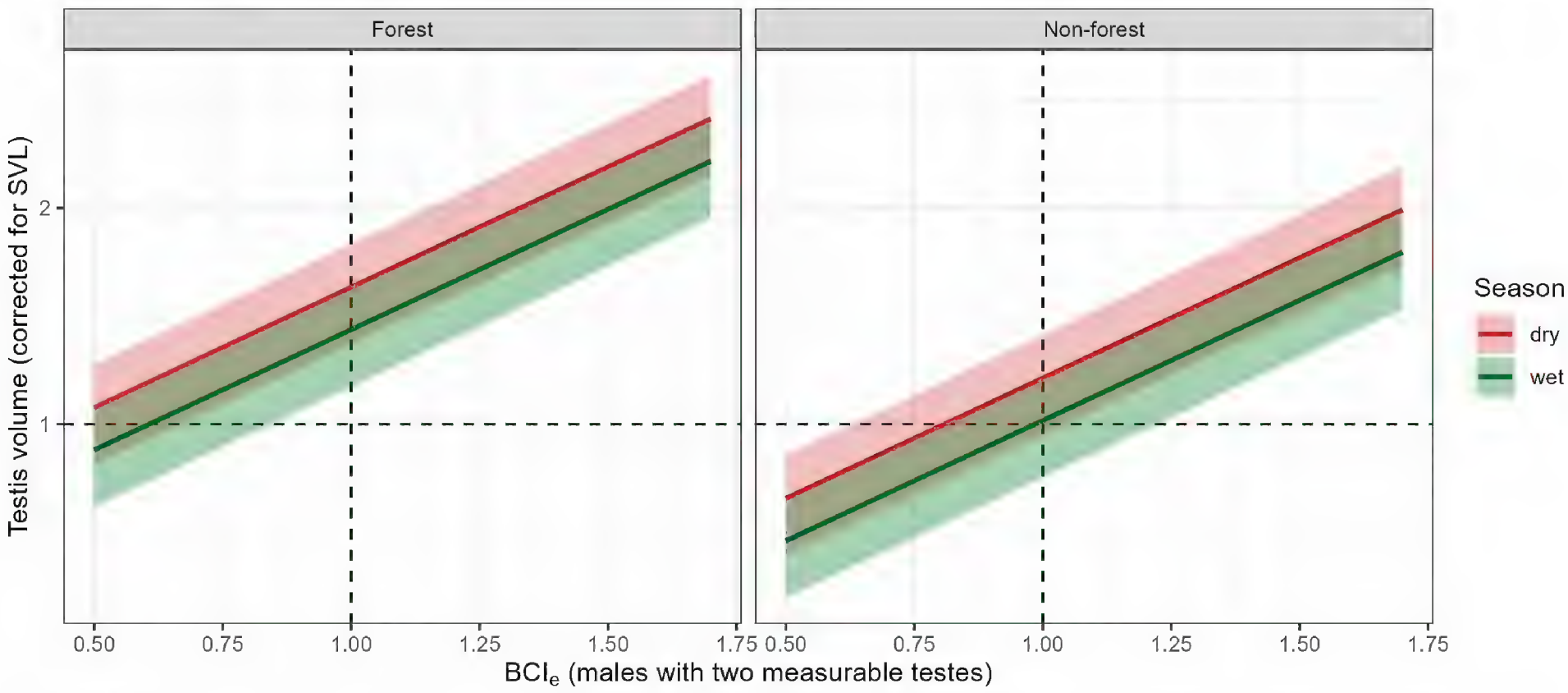


**Figure 12.** Predicted relationships from top mixed linear model between *Boiga irregularis* $BCI_e$ and estimated total testis volume (corrected for SVL) among habitats and seasons.

## Odds ratios and effect sizes

For a one standard deviation increase in BCI, we expect a 329% increase in the odds of a male having two measurable testes (95% CI 46% to 1196%). For a given BCI, the odds of two measurable testes being present increase in the wet season compared to the dry season, but the confidence interval overlaps zero and the effect of season varies by habitat (Table 1; Fig. 11). Compared to ravine forest, we expect

a 71% (95% CI 30–89%) decrease in the odds of males having two measurable testes in limestone forest and the odds of males in urban habitat having two measurable testes are 200% (24–624%) higher (Table 1); confidence intervals for the other three habitats overlap zero.

A one standard deviation increase in BCI was predicted to correspond to an increase (± S.E.) of 1.11 (± 0.30) $mm^3$ in testes volume. For a given BCI, males at forest sites had 0.20 ± 0.08 $mm^3$ more testicular volume, and males in the dry season had 0.42 (± 0.13) $mm^3$ more testicular volume (Table 2).

## Discussion

### Summary

Our findings corroborate previous data on clutch size in the native range (Zwinenberg 1978; Greene 1989; Shine 1991) and provide evidence that BTS reproduction on Guam is mostly aseasonal, that aspects of reproduction are strongly influenced by body condition, and that some variation in reproductive condition among habitats exists, which might be driven by variation among habitats in body condition.

Two aspects limited our ability to make inferences. The first, noted at least since the 1980s, is the relatively low proportion of reproductively mature snakes, especially for a snake species with such a small clutch size (Rodda et al. 1999b). Only 26% of reproductive females (11% of all females) had secondary vitellogenic follicles, slightly higher than other studies (5–22%; Mathies et al. 2001; Waye 2007). The other is that, although we sampled evenly across sites and attempted to stratify sampling by season, our sampling was spread out over two and a half years which led to the capture of reproductive female snakes at variable stages of development. Thus, our measures might not adequately represent female reproductive investment (instead being a snapshot of the current state of reproductive development). For example, analyzing counts of follicles above a specific size assumes that no follicles will be reabsorbed or further enlarged, and analyzing mean follicle size assumes that all follicles have grown as much as they can, all of which are unlikely to be true. The top model from the linear model set with mean follicle length as the response variable contained only BCI. Mean follicle length is probably the most susceptible of our three metrics of female reproductive status to variation in reproductive developmental stage (*i.e.*, some of the variance in mean follicle length among habitats could be due to differences in the timing of sampling, despite our efforts to spread out sampling effort at each site equally between wet and dry seasons). Our linear models consistently had poorer fit than our logistic models, consistent with the idea that the power to detect effects was highest when the response variable was less noisy.

The primary finding for male BTS was that BCI influenced reproductive condition, but variation among sites within habitat types was high enough to prevent making meaningful generalizations. Because population growth and demography are largely driven by females, we focus on female BTS throughout the rest of the discussion.

### Impact of habitat on reproductive condition

Neither the logistic model set nor the linear model set with mean follicle length as the response variable included a habitat scheme in the top-ranked model. The

highest-ranked linear model with follicle count as the response variable included $BCI_e$, season, and the urban-nonurban (UNU) habitat scheme. At the same BCI, females at urban sites were predicted to have 0.28 ± 0.13 fewer follicles than those at non-urban sites (Table 1; Fig. 7A). However, urban female BCI was the highest of any habitat (Figs 3, 4, Suppl. material 1: fig. S2), so mean (± S.D.) clutch sizes were actually higher in urban (5.67 ± 3.20) than in non-urban habitats (4.53 ± 1.75) during the dry season. Clutch sizes were similar in urban (3.38 ± 2.33) and non-urban habitats (3.75 ± 2.58) during the wet season.

Although several aspects of habitat structure could influence BTS reproduction, (*e.g.*, oviposition sites), among the most important is likely to be availability of preferred prey (Rodda et al. 1999b). There are few predators of BTS on Guam (Dryden 1965; Rodda et al. 1999b; Ehrhard 2016). Parasite prevalence, infection intensity, and species diversity are low relative to the native range (Richmond et al. 2012).

BTS have depleted the forests of Guam of their preferred endothermic prey–the endemic community of native forest bird species, which are now mostly extirpated from forests (Savidge 1987; Wiles et al. 2003; Rodda and Savidge 2007). Adult BTS are strongly attracted to bird odors (Fritts et al. 1989; Nafus et al. 2021b) and prey heavily on birds and bird eggs in their native range (Shine 1991). Since c. 1981 adult BTS on Guam have largely shifted from feeding on preferred endothermic prey to feeding on native lizards and on introduced lizards and frogs (Savidge 1988; Rodda and Fritts 1992a; Christy et al. 2007; Siers 2015). Abundant non-native rodents and shrews were probably important drivers of BTS abundance during their early spread, allowing BTS to maintain high population densities and drive native birds to extinction (Savidge 1988; Gragg 2004; Hanslowe 2021). Non-native rodents are still present but at lower densities than on neighboring snake-free islands (Rota, Saipan and Tinian) (Wiewel et al. 2009), and rat density on Guam increased after snake control was applied (Hanslowe 2021), suggesting that BTS continue to suppress mammal populations on Guam (and this may be the case for native lizards as well; Rodda and Fritts 1992a). Likewise, predation by BTS is the major cause of egg and nestling mortality for some species of introduced birds (Conry 1988) and led to the extirpation of at least one introduced bird species (Chestnut Munia, *Lonchura atricapilla*) (Wiles et al. 2003). Introduced bird species include Eurasian Tree Sparrow (*Passer montanus*), Black Drongo (*Dicrurus macrocercus*), Black Francolin (*Francolinus francolinus*), Philippine Collared-Dove (*Streptopelia dusumieri*), and Rock Pigeon (*Columba livia*), as well as captive domestic chickens (*Gallus gallus*). All are consumed by BTS (Conry 1988; Wiles et al. 2003; Vice et al. 2005).

Higher prevalence of larger-bodied endothermic prey species–particularly rodents in savannas and rodents, introduced birds and poultry in urban residential areas–probably improves BTS foraging success in these habitats. Of these, urban rodents and birds are probably the only food sources that are reliably available year-round. However, the forest-savanna-urban (FSU) habitat classification scheme was not highly favored in any model set.

Body condition was highest in urban and lowest in forest habitats for both males and females, and both males and females attained maximum observed sizes in savanna and urban habitats (Siers et al. 2017b). BCI had a stronger effect on reproductive condition response metrics than habitat or season and high BCI in urban habitats might be driving reproduction in those areas. Urban females might have a lower BCI threshold for reproduction. Although body condition thresholds for breeding are known from other snake species (Naulleau and Bonnet 1996), breeding in

semi-arboreal oviparous species might depend more heavily on recent foraging success than in terrestrial viviparous species (Naulleau and Bonnet 1995). Perhaps such a strategy is most viable in urban habitats on Guam, where large, endothermic prey resources are the most abundant and predictable. There are implications for control because body condition affects willingness to ingest carrion, including those containing toxicants, and both traits may have heritability (Levine et al. 2021b; Nafus et al. 2021a). The effect of BCI on susceptibility to trap capture varies among studies (Tyrrell et al. 2009; Lardner et al. 2013; Levine et al. 2019; Levine et al. 2021a).

A snake with poor BCI might still have access to adequate nutrition if it is relying on income breeding and employing a strategy of not "saving for later" (Bonnet et al. 1998). In snakes, different components of reproductive output respond to different temporal scales of prey availability but are functionally linked in ways that likely differ among species (Bonnet et al. 2001).

Snake demography is driven by prey availability in other systems. For example, bottom-up effects of rodent prey population cycles have been shown to drive demography of adders (*Vipera berus*) on Baltic Sea islands (Forsman and Lindell 1991), despite the presence of alternative prey (frogs and bird nestlings). Experimental reduction of rodent prey in savanna resulted in increased BTS movements and an increase in BTS captures in baited traps (Gragg et al. 2007; Christy et al. 2017), suggesting that rodents are important prey and dispersal rates might change in response to changing prey availability.

Evidence from population genetics suggests that both male and female BTS dispersal is random and that gene flow is high, even in urban areas (Kierepka et al. 2019), although like many snakes BTS appear to avoid crossing roads (Siers et al. 2014). On average, BTS move 25–100 m/day and can move among habitats (Santana-Bendix 1994; Feuka et al. 2022). BTS translocated from forests to urban areas survived and their movement patterns exhibited many similarities to those of resident urban snakes (Feuka et al. 2022). Thus, it is conceivable that some BTS might make regular movements to take advantage of spatially-variable resources, such as prey or oviposition sites. High predation rates on fledgling Micronesian Starlings (*Aplonis opaca*) in urban areas (Wagner et al. 2018; Pollock et al. 2019; Pollock et al. 2021) and better body condition and higher female reproductive output of BTS captured at Mariana Swiftlet (*Aerodramus bartschi*) caves support this idea (Klug et al. 2021). The paucity of large prey items in forest habitats (Siers 2015) entails that forest snakes either do not grow as large or robust due to lack of size-appropriate prey or migrate to urban or savanna sites with a greater abundance of these resources, at least intermittently. Although the 18 sites in this study were too widely separated for individual BTS to easily move among them over short time periods, it is possible that gravid female BTS oviposit in different locations than where they spend the rest of their time, although we lack data on movement or oviposition site selection of gravid female BTS. The absence of a connection between mature females in good body condition and proportions of juvenile snakes found at a site (Siers et al. 2017a) represents circumstantial support for this idea. Gravid female BTS do not practice reproductive aphagia (Savidge et al. 2007), so the absence of large post-reproductive females at urban sites could reflect temporary immigration to urban habitats to feed during gestation followed by a return to other habitats for oviposition.

At the same time, snake predation shapes prey communities. In southern Florida, native mammalian prey remain rare 20 years after initial declines caused by invasive Burmese Pythons (*Python bivittatus*) (Dorcas et al. 2012; Redinger et al. 2023),

shifting the community to one primarily composed of invasive species (Taillie et al. 2021). On Aruba, increased reliance on non-native prey allowed an invasive boa (*Boa constrictor*) population to stabilize after a peak (Reinert et al. 2021). Specifically, decreasing prey abundance coincided with the highest boa capture rates and a decline in boa body condition, and boa body condition in residential/agricultural areas averaged 50% greater than in natural areas, evidently due to higher abundance of non-native prey in these habitats (Reinert et al. 2021). However, interaction of these phenomena with reproduction of invasive snakes has been little studied.

In our system, endothermic prey are most abundant in urban habitats, so they could be the most predictable and stable source of prey available. On the other hand, the small ectotherm-dominated prey communities of the forest seem to be abundant year-round (Rodda et al. 2015), although gecko detection rates are lower in limestone forest (Lardner et al. 2015). Still, stability of resource availability is probably an important determinant of reproductive success (Iles et al. 2018). In temperate North America, populations of Wandering Gartersnakes (*Thamnophis elegans*) with stable access to prey exhibit faster growth, earlier maturation, higher fecundity, and lower adult survival compared to populations where prey availability varies annually with presence of standing water (Bronikowski and Arnold 1999).

Limestone forest, the habitat type within which native bird species recovery would be targeted, stands out as having BTS with low BCI (especially for males) and the fewest and most seasonally-clumped reproductive females. This might suggest that BTS in limestone forest are more nutritionally-limited than in other habitats, but can nevertheless persist, ready to take advantage of native forest bird reintroduction should it ever take place (McElderry et al. 2021, 2022). In addition, even when experiencing eradication efforts in the Northwest Field North (NWFN) limestone forest enclosure, where BTS cannot rely on resource subsidies from adjacent habitats, mature females are able to sustain a population through reproductive bursts (Nafus et al. 2024).

## Impact of season on reproductive condition

Evidence for seasonality is present but the extent of seasonality might vary among habitats. The raw proportions of mature females with secondary vitellogenic follicles are higher in the dry season (December-May; 48%) than in the wet season (June-November; 30%) (Suppl. material 1: table S1), and logistic models predicted a higher probability of mature females having secondary vitellogenic follicles in the dry season compared to the wet season (Table 1, Fig. 6). This takes into account variation in BCI and among sites, but the modest effect size compared to that for BCI (Table 1) serves to reinforce the absence of strong seasonality in BTS reproduction. Urban habitats were the only habitats with reproductive female BTS in all months, consistent with the idea that urban habitats might be better able to support continual reproduction than forest habitats. Our data also suggest that female reproductive investment might be concentrated in April-June in limestone forest to a greater extent than in other habitats. Waye (2007) found that female BTS on Guam become vitellogenic in the latter part of the dry season and into the wet season but did not report the habitat of the BTS they studied and concluded that female BTS reproduction on Guam might extend from March to November. Based on the presence of shelled eggs in necropsies and oviposition dates of recently-captured females, eggs can be laid at least from May to October (McCoid 1994; Linnell et al. 1997; Waye 2007).

Follicle counts were slightly higher in the dry season in most habitats (Figs 4C, 7A), especially in Leucaena stand, scrub forest, and urban. The effect of season is small compared to that of BCI, and there could be an interaction among season, BCI, habitat and reproduction. BCI distributions tended to deviate from average in the dry season, although direction varied by habitat and sex (Siers et al. 2017b). Also, although we attempted to stratify sampling by season, balance was not as easily achieved as among habitats.

In thermally stable tropical climates, rainfall varies seasonally whereas mean temperature is stable throughout the year (Rodda et al. 1999a). Counterintuitively, reproductive cycles of many tropical snake species are seasonal and synchronous among individuals, as in temperate zones (Duvall et al. 1982; Vitt and Vangilder 1983; Shine 1985). However, many species of tropical snakes are reproductive for extended periods and some apparently reproduce year-round (Saint Girons 1982; Mathies 2011). The apparent absence of a non-breeding season among BTS on Guam poses a challenge to control efforts because breeding snakes are the least detectable and continual recruitment may necessitate continual management to achieve suppression (Rodda and Savidge 2007).

## Conclusions

Overall, body condition was by far the most important predictor of whether female BTS were reproductive, followed by season, followed by urban habitat (Table 2). Our findings provide circumstantial evidence that female BTS might support reproduction by feeding on introduced prey, such as rats, domestic chickens, tree sparrows, and green anoles (*Anolis carolinensis*), in urban and savanna habitats. This could be considered an example of a "cross-boundary" subsidy (Luskin et al. 2017), similar to when wildlife enter agricultural areas to raid crops or depredate livestock. The ectotherm-dominated prey communities of Guam's forests might sustain individual BTS, but it seems likely that dependable access to large, endothermic prey more readily fuels reproduction. The relative roles of plasticity and adaptation acting on growth rate, age at maturity, fecundity, and adult survival of BTS on Guam are not entirely clear, though it does not appear that BTS have particularly high reproductive output for a snake and might achieve their high population density through high survivorship rather than exceptional fecundity (Rodda and Savidge 2007).

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

## Ethical statement

All animal use was conducted in accordance with Colorado State University Institutional Animal Care and Use Committee (IACUC) Protocol #09-1436A.

## Use of AI

We did not use AI for any aspect of the paper. All data were collected before AI was commonly available. I affirm that all analysis and writing was done by humans.

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## Author contributions

AMD conducted analyses and wrote the paper. SRS collected the data, oversaw field personnel, generated the idea as part of his dissertation work, and acquired funding for and collaborated on the contents and structure of the analyses and paper. RNR and JAS co-advised SRS's dissertation, acquired and administered funding for, and oversaw all work. All authors contributed to revising the article. AMD and SRS contributed equally and should be considered co-first authors.

## Author ORCIDs

Andrew M. Durso https://orcid.org/0000-0002-3008-7763
Shane R. Siers https://orcid.org/0000-0001-7961-5072
Robert N. Reed https://orcid.org/0000-0001-8349-6168
Julie A. Savidge https://orcid.org/0000-0002-7995-0679

## Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Supplementary material 1

### Supplementary figures and tables

Authors: Andrew M. Durso, Shane R. Siers, Robert N. Reed , Julie A. Savidge

Data type: docx

Explanation note: **fig. S1.** Variation in A) number and B,C) average length (mm) of secondary vitellogenic follicles (those ≥5 mm) compared to female *Boiga irregularis* snout-vent length (SVL); **fig. S2.** Comparison of $BCI_a$ among six habitat types for A) female and B) male *Boiga irregularis*; **fig. S3.** Assessment of model fit for top mixed logistic model between $BCI_a$ and probability of enlarged (≥5 mm) follicles being present in female *Boiga irregularis* in wet and dry seasons (predicted values shown in Fig. 6); **fig. S4.** Assessment of model fit for top linear mixed models between $BCI_e$ and follicle count (corrected for SVL) between seasons and urban and non-urban habitats in female *Boiga irregularis* (predicted values shown in Fig. 7A); **fig. S5.** Assessment of model fit for top linear mixed models between $BCI_e$ and mean follicle length (corrected for SVL) in female *Boiga irregularis* (predicted values shown in Fig. 7B); **fig. S6.** Assessment of model fit for top mixed logistic model between $BCI_a$ and probability of male *Boiga irregularis* having two measurable testes among habitats and seasons (predicted values shown in Fig. 11); **fig. S7:** Assessment of model fit for top mixed linear model between $BCI_e$ and estimated total testis volume (corrected for SVL) among habitats and seasons for male *Boiga irregularis* (predicted values shown in Fig. 12). All plots were generated using package DHARMa. **table S1.** Proportion of female *Boiga irregularis* captured that had enlarged follicles (≥5 mm) in each habitat in each month (years pooled). **table S2.** Model selection table for mixed logistic regression with presence/absence of secondary vitellogenic follicles (≥5 mm) as the response variable and site as a random effect. **table S3.** Model selection table for mixed linear regression with *Boiga irregularis* secondary vitellogenic follicle count (corrected for SVL) as the response variable and site as a random effect. **table S4.** Model selection table for mixed linear regression with *Boiga irregularis* mean secondary vitellogenic follicle length (corrected for SVL) as the response variable and site as a random effect. **table S5.** Proportion of male *Boiga irregularis* captured that had visible testes in each habitat in each month (years pooled). **table S6.** Model selection table for mixed logistic regression with presence/absence of two measurable testes in male *Boiga irregularis* as the response variable and site as a random effect. **table S7.** Model selection table for mixed linear regression with *Boiga irregularis* estimated testis volume (corrected for SVL) as the response variable and site as a random effect.

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